Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander per et al. Express Mail No. EL897868473

Docket No. 210121.465C6

HU: MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLMO: MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL

HU: GGPAPPPAPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG MO: GGPAPPPAPPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG

HU: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS MO: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG MO: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG

HU: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE MO: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE

HU: GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS MO: GQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS

HU: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRMO: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU: SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR MO: SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR

HU: WPSCQKKFARSDELVRHHNMHQRNMTKLQLAL MO: WHSCQKKFARSDELVRHHNMHQRNMTKLHVAL

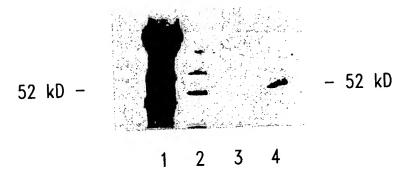


Fig. 2

Express Mail No. EL89786847

Docket No. 210121.465C6

IOOUESUS IUSUUI

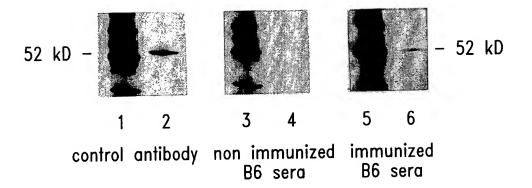


Fig. 3

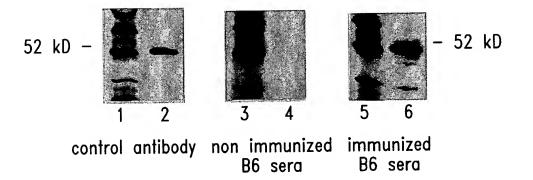
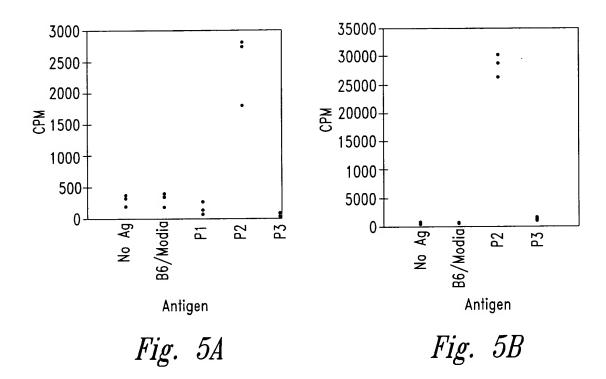


Fig. 4



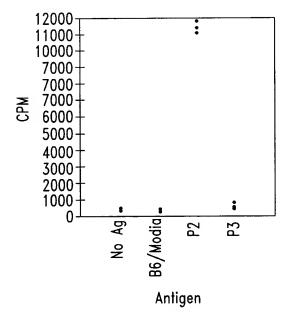


Fig. 5C



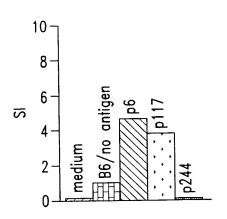


Fig. 6A

Vaccine B stimulated line

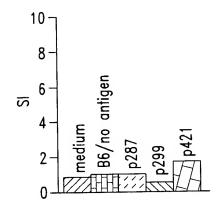
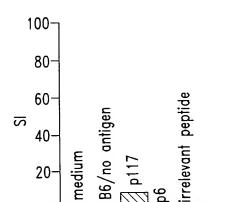


Fig. 6B

p117-139 stimulated line



20

Fig. 7A

p117-139 stimulated clone

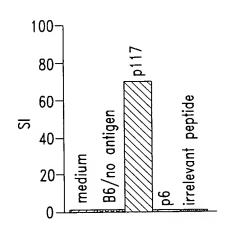


Fig. 7B

p6-22 stimulated line

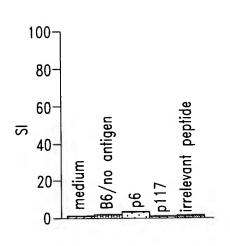


Fig. 7C

p6-22 stimulated clone

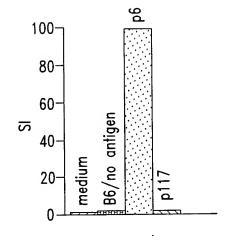


Fig.

5 MGSDVRD AA	LNALLI	PAVPSI WAAAA	_GGGG(GCALP'	VSGAA(AAA/ RRRF	QWAPVI VAA R	LDFAPI	PGASA . AAA	YGSLG Vaaaa 	GPAPP VAAA . 	PAPPP 		PHSFIK 	
80 PSWGGAE	85 PHEEQ	90 CLSAF AAA	95 TVHFS \	100 GQFTG AAA R	105 TAGAC A RRR	110 RYGPF	115 GPPPP	120 SQASS	 125 GQARM AAA RR DDD	130 FPNAP	 135 YLPSC .AAAA	140 CLESQP AA	145 1 AIRNQO	150 GYS
TVTFDG	160 TPSYGH	165 ITPSHH A R	170 IAAQFF AAAA . RRR	175 PNHSFK	180 (HEDPN	185 1GQQGS	190 SLGEQO	195 (YSVPF	200 PPVYG(A	205 CHTPTI AAAA/	210 DSCTG: A	215 SQALLL 	220 RTPYS	225 SDN . AA
LYQMTS AAAAAA DDDDDD	235 QLECMT AA	240 [WNQMN	245 Ilgati A 	250 LKGVA/ AA . AA RRRRF	255 AGSSSS A RR	260 SVKWTI .RRRF D	265 EGQSNH 	270 HSTGYI	275 ESDNH	280 TTPIL	285 CGAQY 	290 RIHTH(A A F	295 GVFRGI AAAAAA RRRR	300 QDV VAAA
RRVPGV AAAAA RR	310 'APTLV . AAAA RRR . DDDDI	315 RSASE AAAAA DD	320 TSEKR AA	325 PFMCA 	330 YPGCN	335 KRYFK .RRRR	340 LSHLQ 	345 MHSRK 	350 HTGEK	355 PYQCD	360 OFKDCE	365 ERRFSR WAA . A	370 SDQLKF AAAAA	375 RHQR AAA.
380 RHTGVI	385 (PFQCK	390 TCQRK AAA	395 FSRSE A . AAA 	400 OHLKTH AAA	405 HTRTHT	410 GKTSE AAAA.	415 EKPFSC	420 :RWPS0 A	425 CQKKF/ VAA RRR	430 Arsdei . Aaa/ Br Rf	435 VRHHI VAAAA RRR	440 NMHQRN AAA	445 IMTKLQI	450 LAL

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 PSWGGAEPHEEQCLSAFTLHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYS AAAA
RRRR DDDDDDDDD
155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 TVTFDGAPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN AAAAAA
230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 LYOMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTEGQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDV AAAAAAAAA AAA. AAA. RRRRRRRRR RRR. RRRR. DDDDDDD DDDDDDDDDD
305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 RRVSGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQRAAAA .AAAAAAAAAARRRRRRRR
380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWHSCQKKFARSDELVRHHNMHQRNMTKLHVAL AAAA.AAAAAAAAAA
dddddddddd

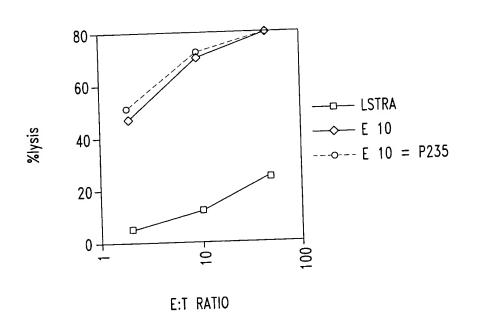


Fig. 9A

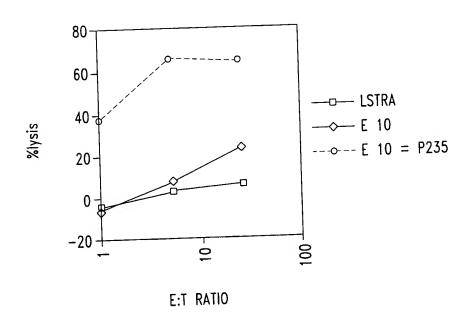


Fig. 9B

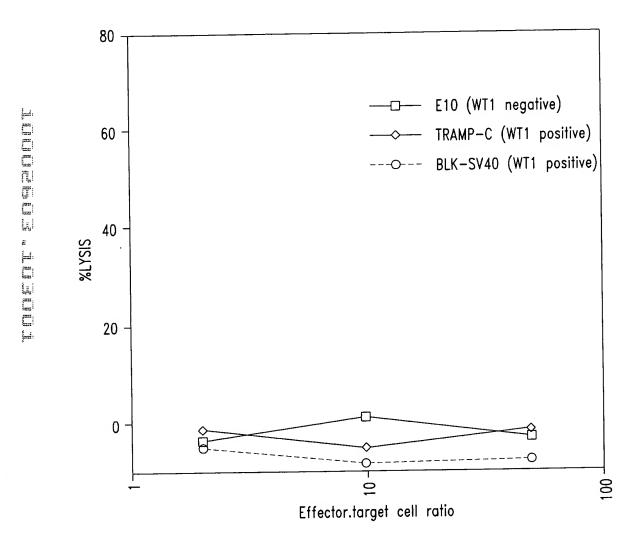


Fig. 10A

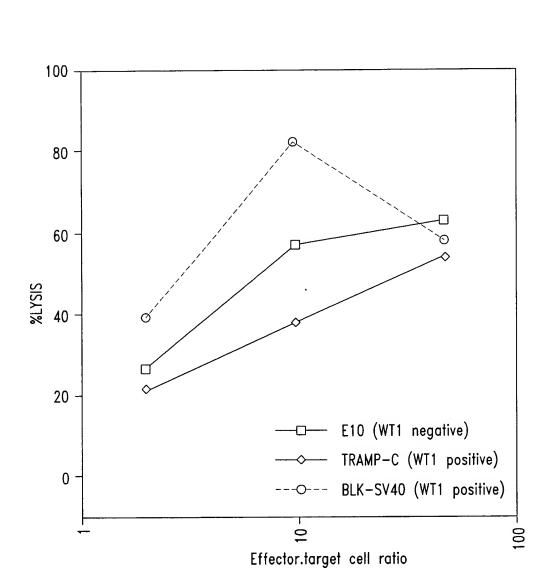


Fig. 10B



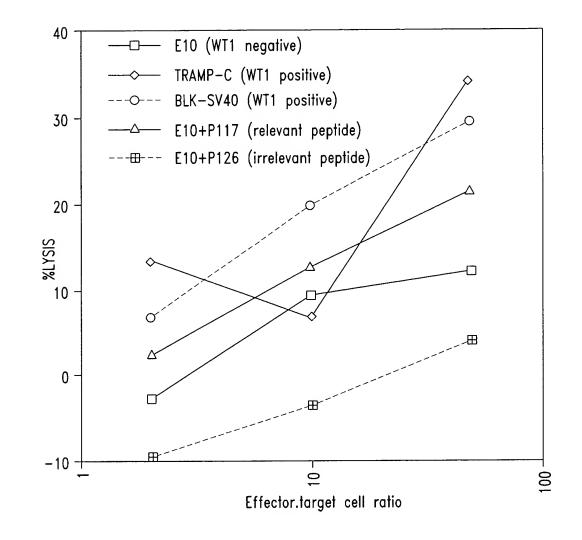
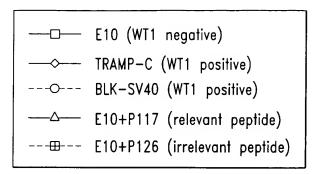


Fig. 10C



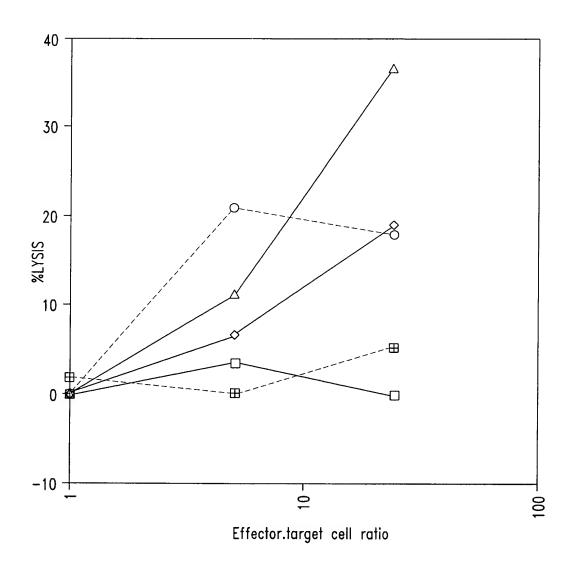
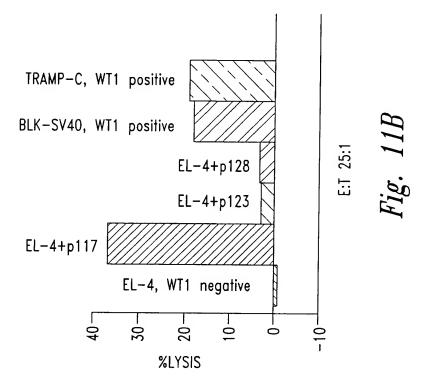


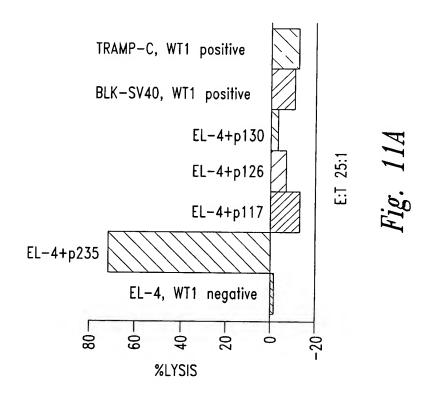
Fig. 10D

ger et al.

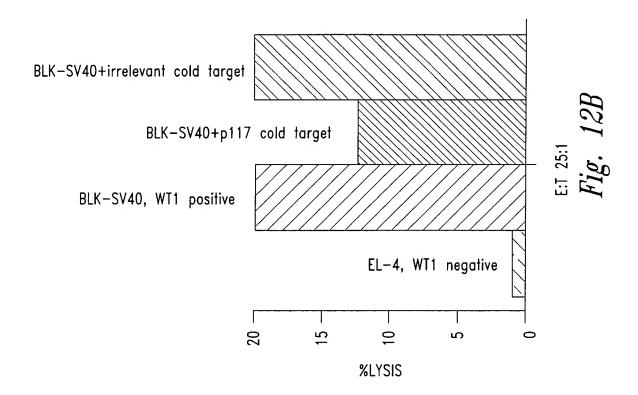
Express Mail No. EL89786847

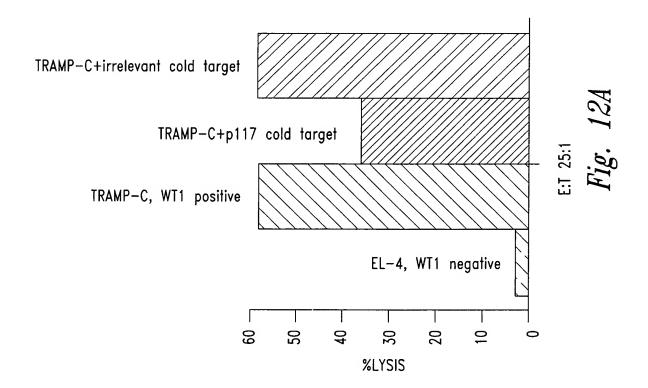
Docket No. 210121.465C6

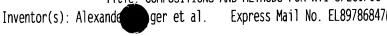




IJUGEBUS JUSUU1







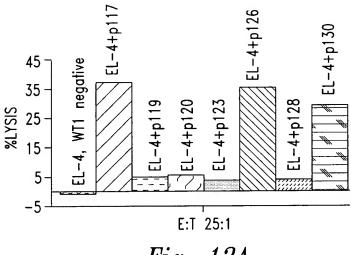


Fig. 13A

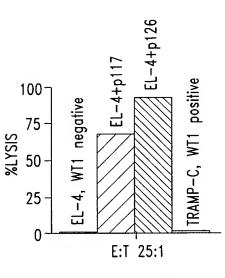


Fig. 13B

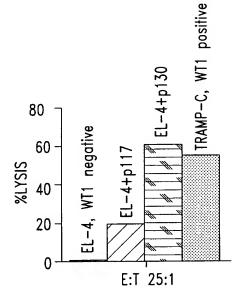


Fig. 13C

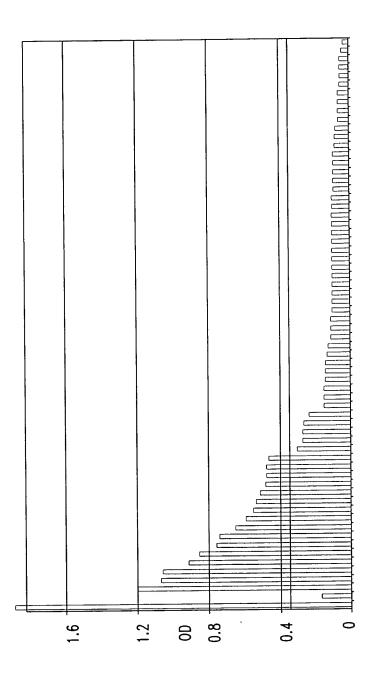


Fig. 14

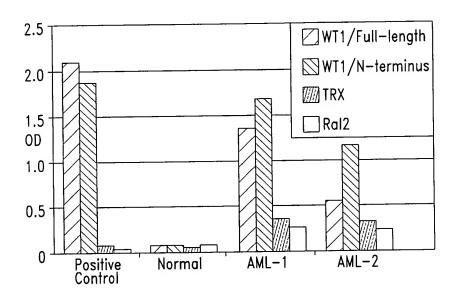
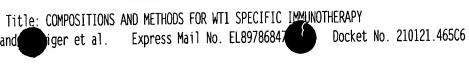
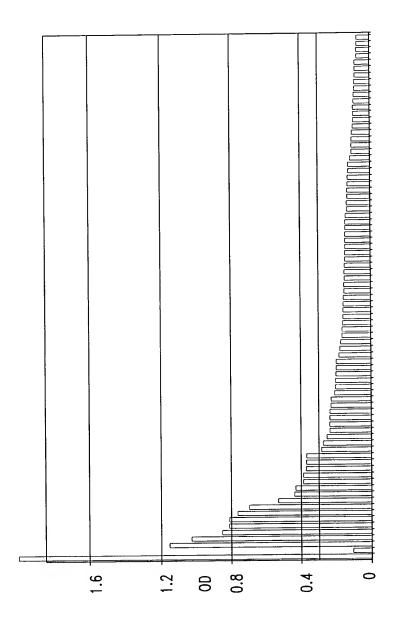


Fig. 15





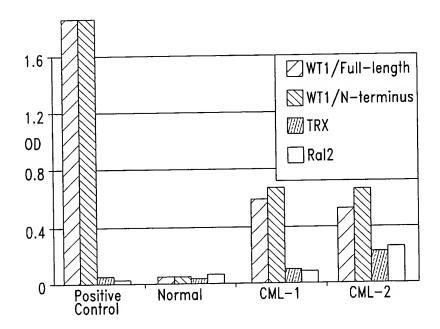


Fig. 17

Molecular Weight 85kDa 60kDa 50kDa

ALGUEBUB ALGEGUA

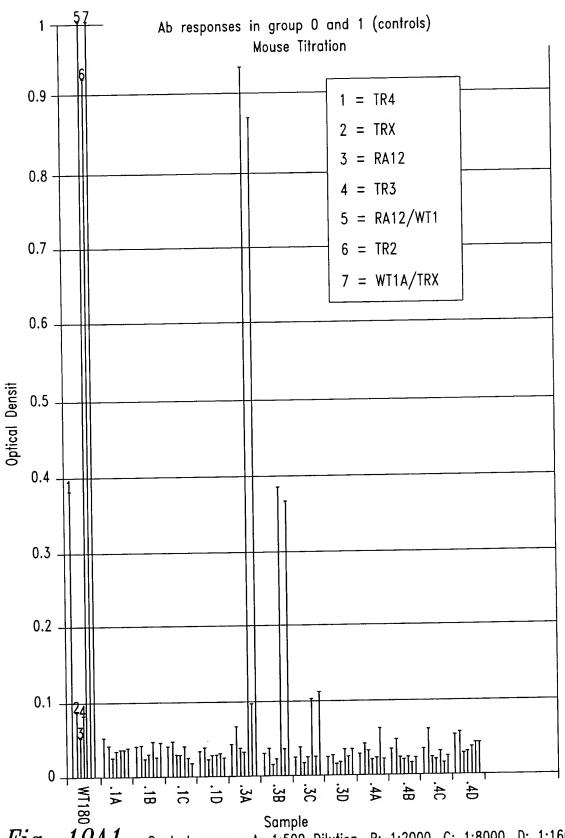
TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

Recombinant Protein	Ral2—WT1 full length fusion protein	TRX-WT1 N-terminus fusion protein	W11 C-terminus protein
NAME	WT1/full-length	WT1/N-terminus	WT1 /C-terminus

1 Position			
Acid			0
Amino	1-449	1 - 249	767-449
WT	8	8	5

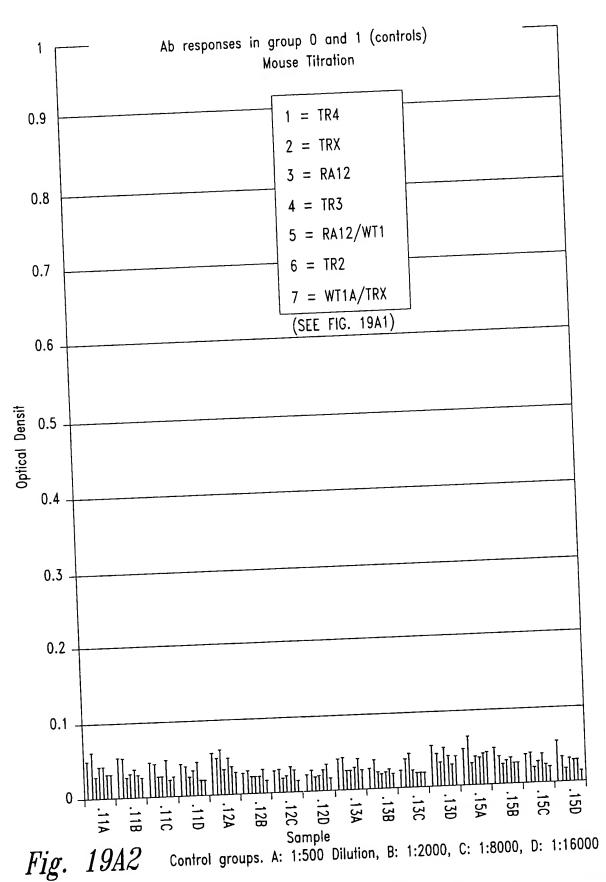
Fig. 18

Docket No. 210121.465C6 Express Mail No. EL89786847 Inventor(s): Alexand iger et al.



Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000







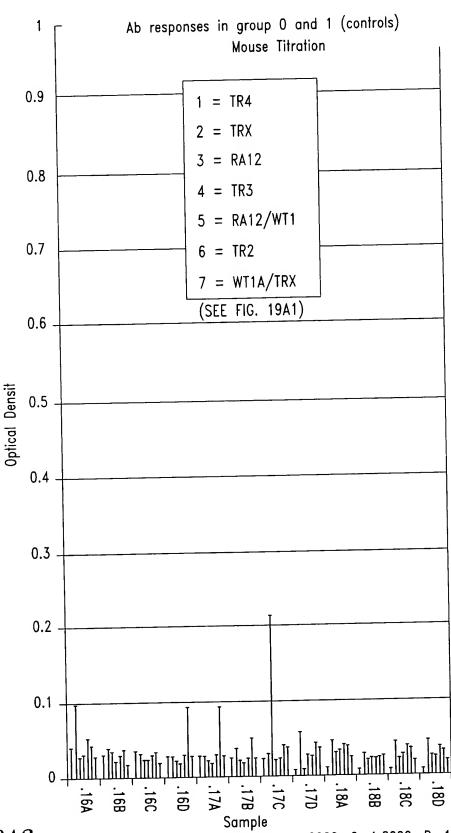
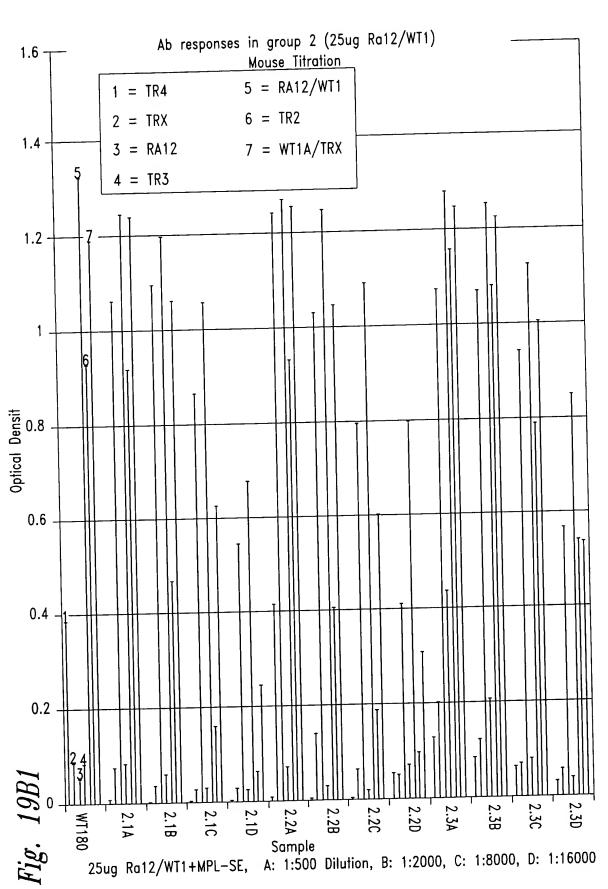


Fig. 19A3 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

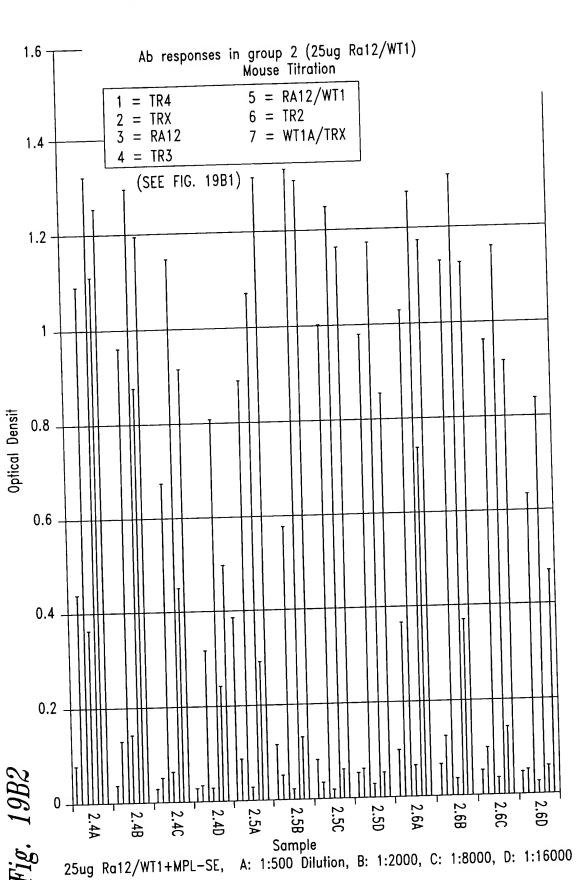
Express Mail No. EL89786847

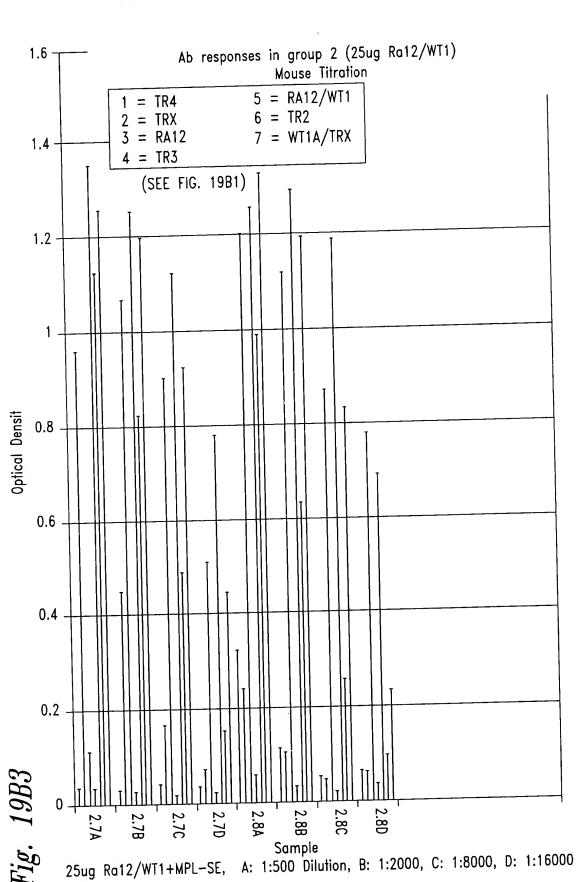
Docket No. 210121.465C6

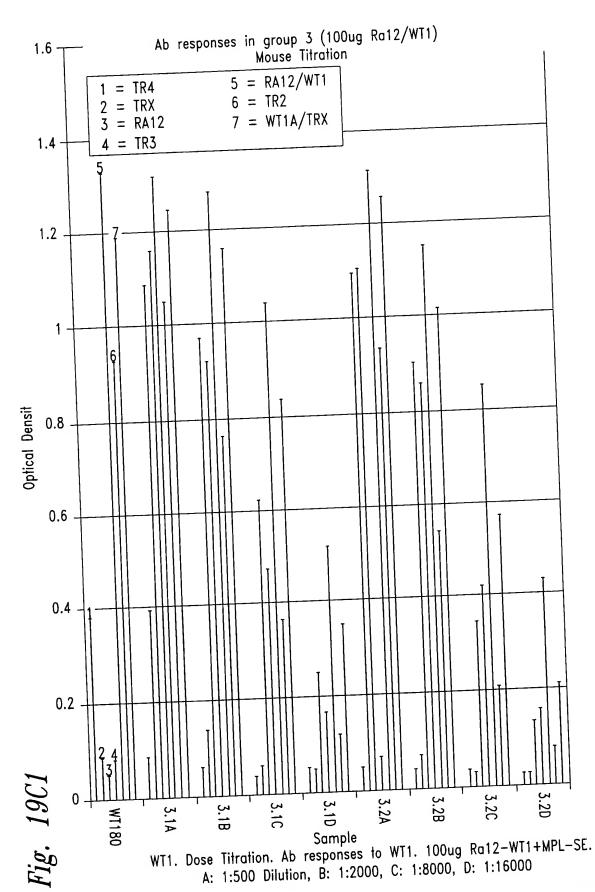


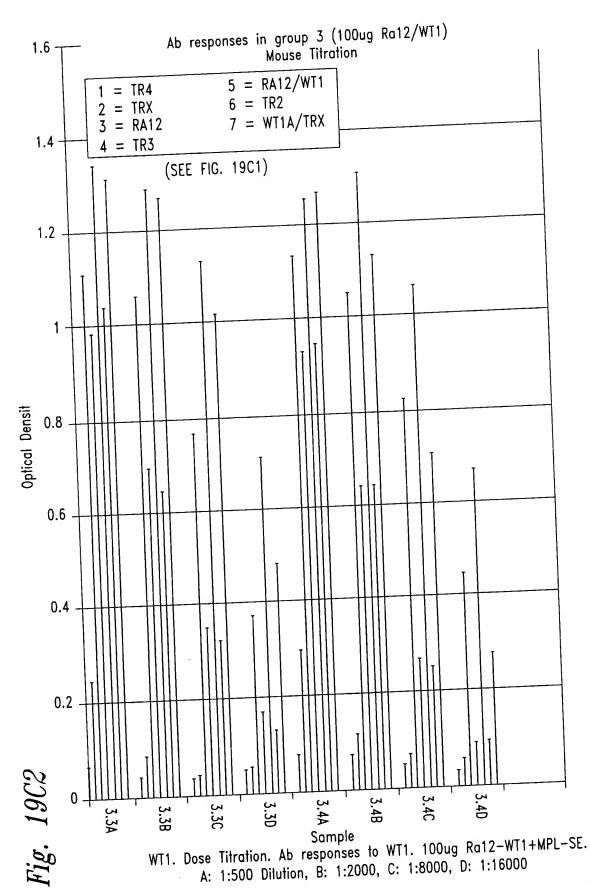




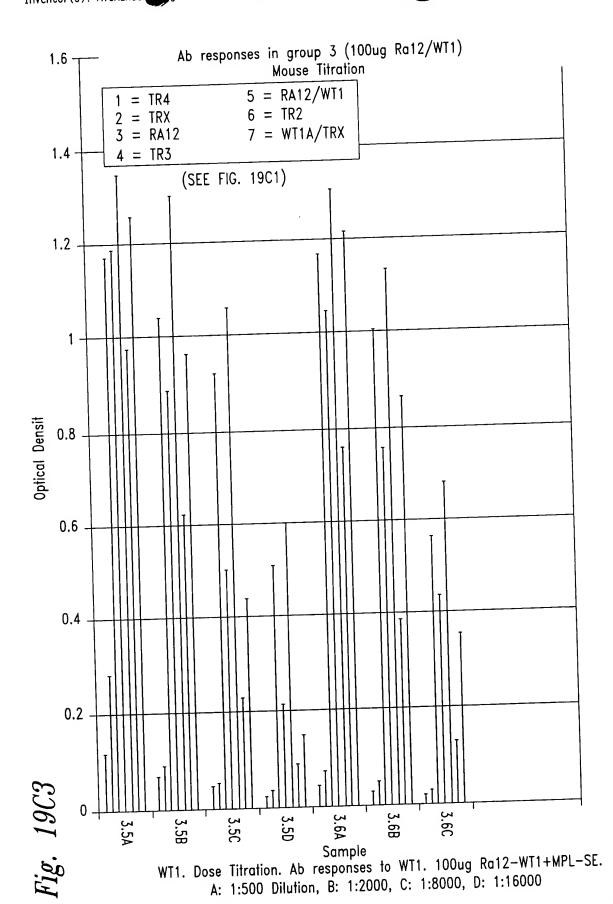




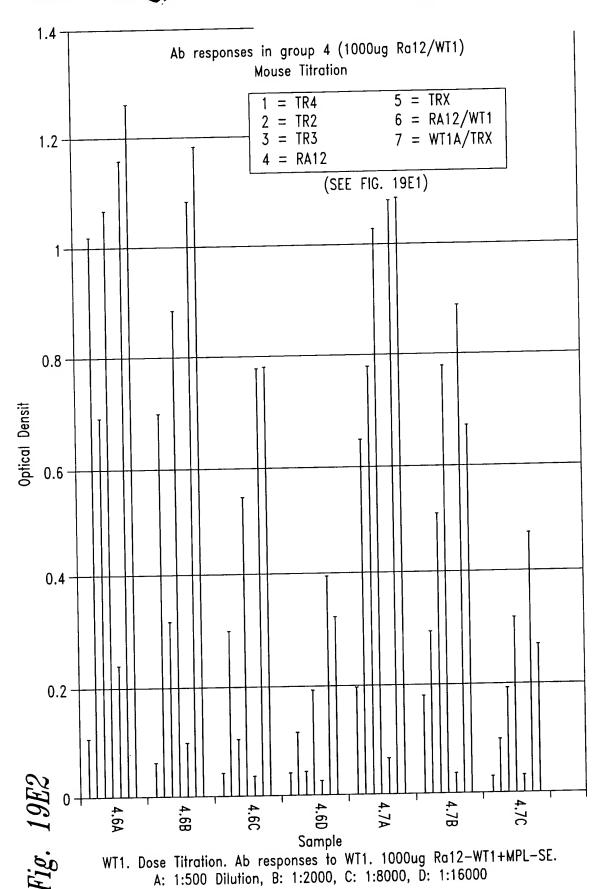






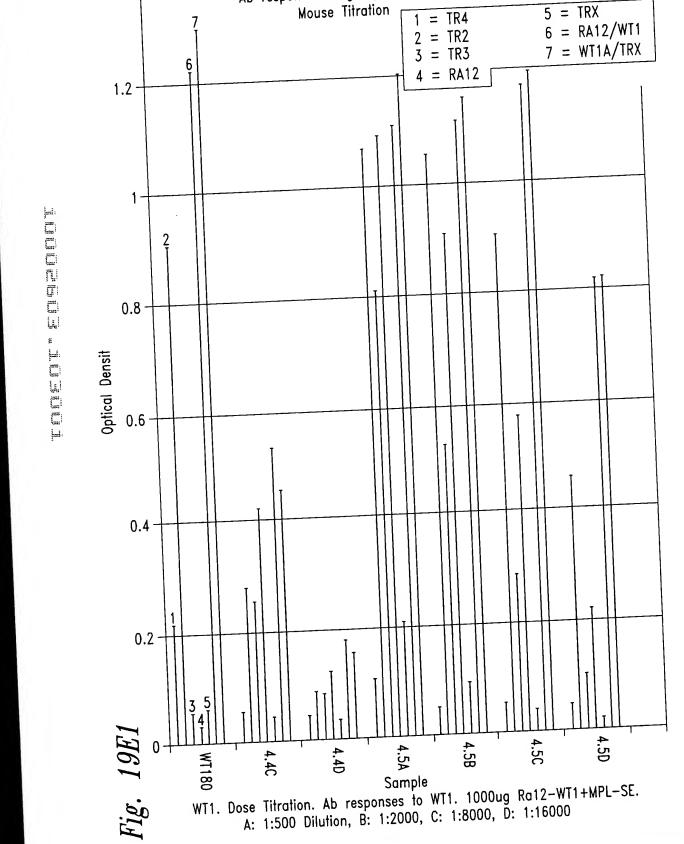




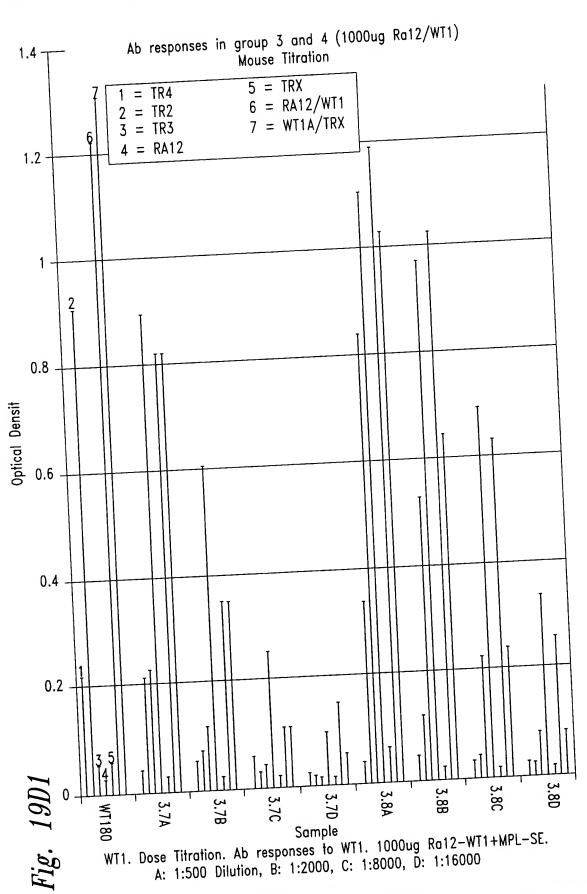


Ab responses in group 4 (1000ug Ra12/WT1)

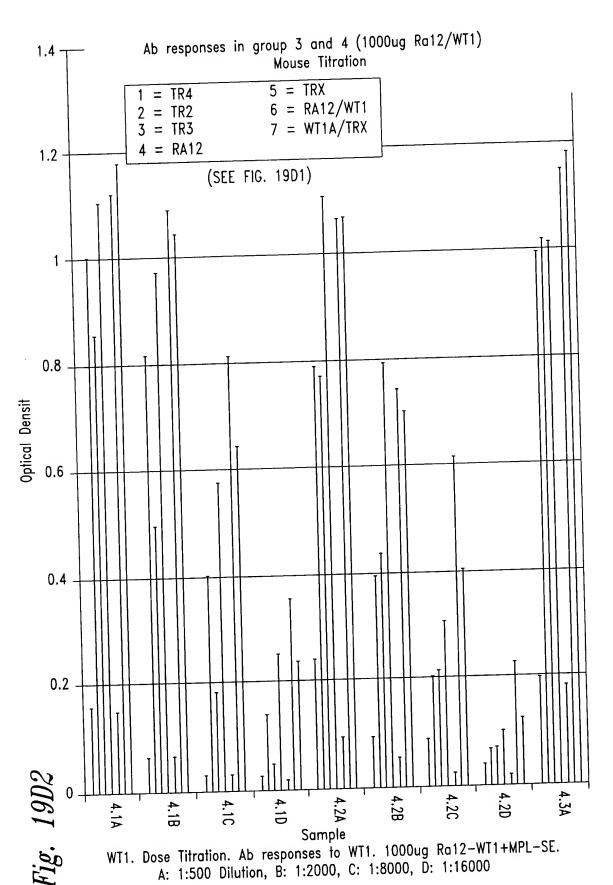
1.4

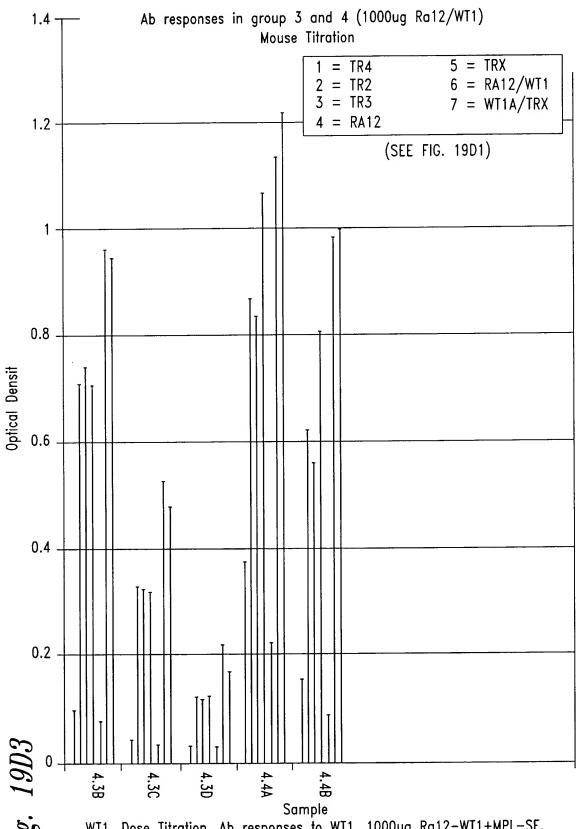


Sample WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



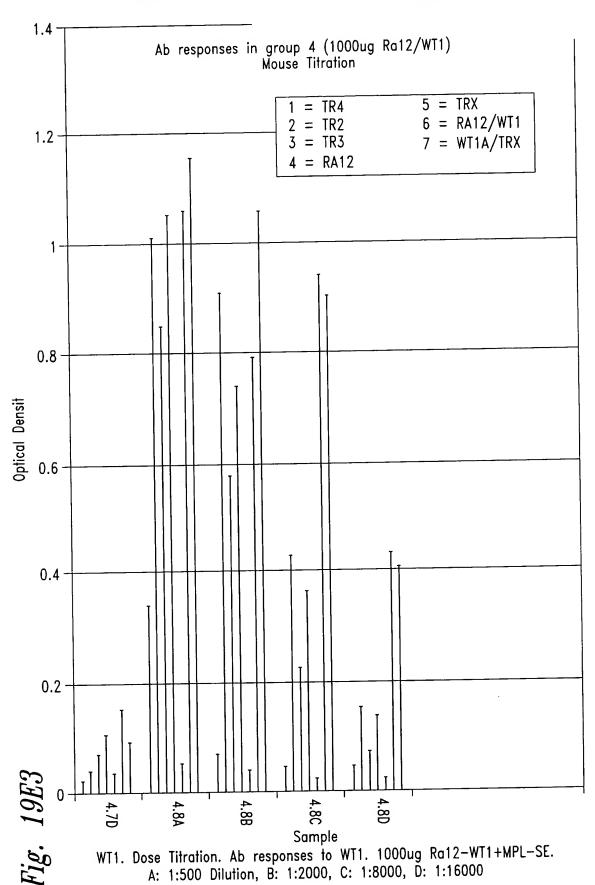


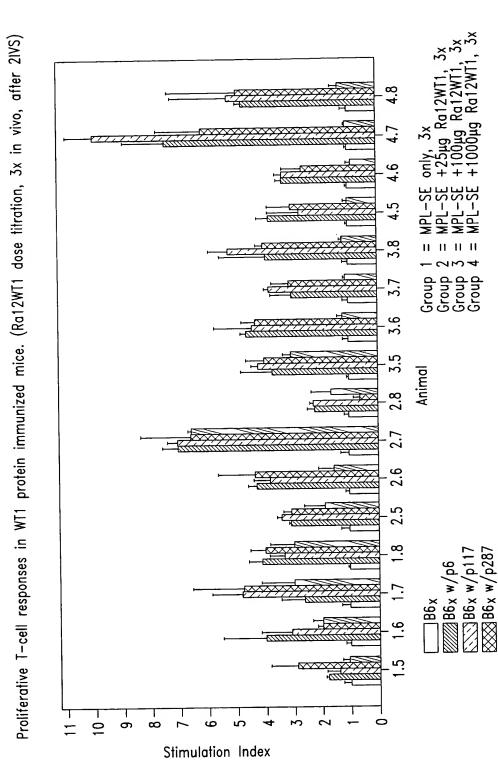




WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



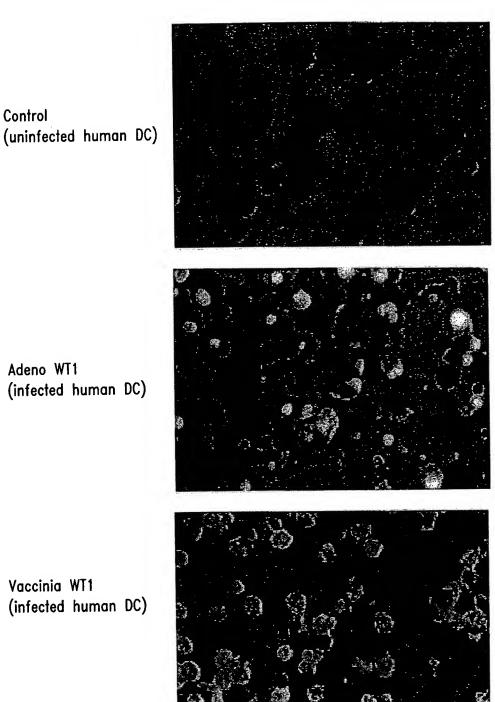




Proliferative T—cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 6x in vivo, after 2IVS) Group Group Group Group Animal 86_× ٨. 0 7 ~

Stimulation Index

WT1 expression in human DC following adeno WT1 and Vaccinia WT1 infection



Adeno WT1 (infected human DC)

Control

Vaccinia WT1 (infected human DC)

Fig. 21

WT1 can be expressed reproducible in human DC following adeno WT1 infection and is not induced by a control Adeno infection

Control (Adeno EGFP infected human DC)



Vaccinia WT1 (infected human DC)

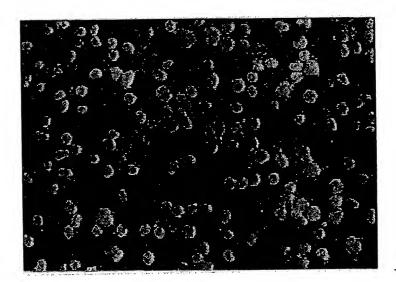
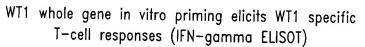


Fig. 22



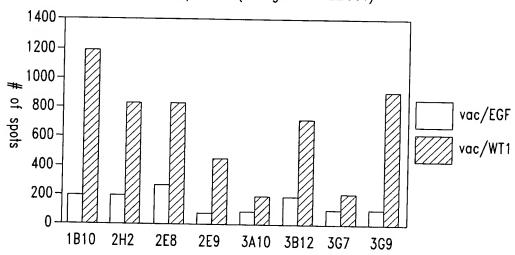


Fig. 23